Metabolic Fate of SK-896, a New Human Motilin Analogue ([Leu^{13}]motilin-Hse) (II): Blood and Plasma Concentration and Excretion in Rats after Single Intravenous Injection or Constant Rate Intravenous Infusion of ^3H-SK-896

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Summary: SK-896 ([Leu^{13}]motilin-Hse) is a new human motilin analogue synthesized by Escherichia coli using a biotechnological method. In this study, the pharmacokinetic properties of SK-896 after a single intravenous injection or during and after a single constant rate intravenous infusion of rats with ^3H-SK-896 were assessed. After intravenous bolus injection of male and female rats with ^3H-SK-896 at the dose of 4 µg/kg, the plasma levels of immunoreactive radioactivity declined bi-exponentially. No sex difference in pharmacokinetic properties was observed after the intravenous bolus injection. During constant rate intravenous infusion of male rats at the dose of 4 µg/kg/h for 20 min, the plasma level of immunoreactive radioactivity increased rapidly, and declined bi-exponentially after the completion of infusion. No significant differences in pharmacokinetic parameters were observed between intravenous bolus injection and intravenous infusion, suggesting that there was essentially no change in the pharmacokinetics by the administration rate. Within 120 h after intravenous bolus injection to male and female rats at the dose of 4 µg/kg, 10.9%, 9.55% and 3.84% of administered radioactivity were excreted into urine, feces and expired air in males, against 22.7%, 13.1% and 5.38% in female rats, respectively. Serum protein binding of ^3H-SK-896 was 91.4–94.2% in dog, 93.6–96.6% in rat and 96.2–97.0% in human. Binding ratio of ^3H-SK-896 to human serum albumin was 84.2–87.0%. Concentration-dependency of ^3H-SK-896 binding to serum proteins was not observed over the concentration range studied.

Key words: SK-896, [Leu^{13}]motilin-Hse, Pharmacokinetics, Excretion, Protein binding, Rat

Introduction

SK-896 (Phe-Val-Pro-Ile-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Leu-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-Hse), in which leucine replaces methionine at position 13 of human motilin and homoserine is added at the C-terminal, is a new human motilin analogue. A pharmacological profile of SK-896 reported in rabbits, rats and dogs using in vitro techniques, revealed that it bound the motilin receptors in the rabbit duodenum and induced contraction of the smooth muscle preparation isolated from the rabbit gastrointestinal tract but not that isolated from the rat or the dog. In addition, in the in vivo study, it was reported that treatment with SK-896 significantly shortened the time of first appearance of interdigestive migrating contractions in stomach and the gastric emptying time with solid marker in dogs with operative ileus as compared with treatment with prostaglandin F_{2α}, which is currently used to treat of gastroparesis. SK-896 has the same pharmacological profile as human motilin in vitro, and is more effective than prostaglandin F_{2α} in vivo. Clinically, intravenously administered motilin or motilin analogue enhances GI motility without serious side effects, indicating that SK-896 will be more useful and effective for the treatment of gastroparesis after abdominal surgery and for diabetes.

The aim of this study was to investigate the pharmacokinetics of SK-896 after single intravenous administration of ^3H-SK-896 in rats.

Materials and Methods

1. Materials

SK-896 ([Leu^{13}]motilin-Hse) was synthesized at Sanwa Kagaku Kenkyusho Co., Ltd. ^3H-SK-896 (Tyr-3,5-^3H; Phe-Val-Pro-Ile-Thr-Tyr-3,5-^3H)-Gly-Glu-Leu-Gln-Arg-Leu-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-Hse, 325.8 MBq/mg, 97.7%) was purchased from Daiichi Pure Chemical Co., Ltd. Rabbit anti-human motilin antiserum was prepared by our laboratory. The antiserum was shown to react mainly with the C-terminal portion of SK-896 but never with other known gastrointestinal hormones. Aprotinin (Sigma Chemical Co.), Tween 80 (Katayama Chemical Industries, Ltd, Tokyo, Japan), albumin bovine fraction V (Sigma Chemical Co.), and albumin human fraction V (Sigma Chemical Co.) were used. All other chemicals were of reagent grade.
2. Animals
Sprague-Dawley (SD) rats (SLC Inc., Shizuoka, Japan), 7 weeks old and weighing 226-238 g (male) and 162-173 g (female) were used. The animals were housed in an air-conditioned room at 22 ± 3°C with relative humidity of 55 ± 15% and a 12-h light cycle, fed standard laboratory diet (CRF-1, Oriental Yeast, Co., Ltd., Tokyo, Japan) and given water ad libitum. The animals were fasted overnight prior to administration.

Also, male and female Beagle dogs (Kitayama Labes company Ltd., Kyoto, Japan), 22 and 38 months old, weighing 9.3 and 8.2 kg were used for the protein binding study.

3. Subjects
Two healthy male subjects, 35 and 38 years of age, participated in protein binding study after giving their informed consent.

4. Preparation of Dosing Solution
The dosing solution was prepared by diluting an appropriate amount of ³H-SK-896 stock solution (200 µg/ml) with physiological saline. ³H-SK-896 was diluted by non-labeled SK-896. The radiochemical purity of the dosing solution was confirmed according to the methods in a previous report.5)

5. Pharmacokinetic Studies
Male and female rats were given an intravenous bolus of ³H-SK-896 at 4 µg/kg (1.30 MBq/kg) via the tail vein. Blood samples (0.06–0.22 ml) were collected on an EDTA anticoagulant via the jugular vein at 2, 4, 6, 8, 10, 15, 20, 30, 40 min, 1, 2, 4, 6, 8, 24 and 48 h after administration. After aliquots of blood were measured, plasma was immediately separated from blood by centrifugation and stored at −80°C until analysis.

Male rats were intravenously infused with ³H-SK-896 at a dose of 4 µg/kg/h (1.30 MBq/kg/h) over 20 min. The administration was done with an indwelling needle (24 G × 3/4") in the femoral vein, and the infusion rate was set at 0.021 ml/min. Blood samples (0.06–0.22 ml) were collected at 5, 10, 15, 19, 22, 24, 26, 28, 30, 35, 40, 50 min, 1, 2, 4, 6, 8, 24 and 48 h after starting the infusion.

For determination of immunoreactive radioactivity in plasma, ³H-SK-896 was administered to separate groups of rats at 4 µg/kg (1.30 MBq/kg) for intravenous bolus injection and at 4 µg/kg/h (1.30 MBq/kg/h) over 20 min for intravenous infusion. In these groups, aprotinin was added to blood for a final concentration of 1000 kallikrein inactivator units (KIU) /ml.

Furthermore, because there were many blood collecting points, the hematocrit values of all rats were measured in the final blood collecting point. It was confirmed that the hematocrit value did not decrease significantly.

6. Excretion Studies
Male and female rats were housed individually in glass metabolic cages (Metabolic®, Sugiyamagen Iriki, Tokyo, Japan) after intravenous bolus injection of ³H-SK-896 at 4 µg/kg (0.65 MBq/kg). Urine, feces and expired air were collected separately up to 120 h. For determination of immunoreactive radioactivity in urine, the urine until 6 h was collected, cooled by ice, and aprotinin was added beforehand to exceed the final concentration of 1000 KIU/ml. Each urinary sample was diluted 20 to 50 ml with distilled water. Fecal samples were homogenized with distilled water after weighing. The expired air was collected by two traps containing about 90 ml of distilled water. The trapped water was diluted to 200 ml with distilled water. After collecting excreta until 120 h, the rats were sacrificed by etherization and the carcasses were solubilized by heating and refluxed in 400 ml of 0.5 N sodium hydroxide and 70 ml of toluene. The water layer of the carcass solution was diluted to 1000 ml with distilled water after neutralization with conc. hydrochloric acid. The toluene layer of the carcass solution was diluted to 100 ml with toluene.

7. Protein Binding Studies
In vitro bindings of ³H-SK-896 to plasma protein in rats, dogs and humans and human serum albumin (HSA) were determined by the charcoal-dextran precipitation method. ³H-SK-896 was added to the sera or HSA at the range of 1.0–1000 ng/ml (rats, dogs and 4.0% HSA) and 1.0–100 ng/ml (humans). After incubation of 300 µl of samples for 15 min at 37°C, 1000 µl of 0.13 M phosphate buffer (pH 7.4) containing 1.5 mg/ml charcoal, 0.15 mg/ml dextran T-70, and 0.1% bovine serum albumin was added and stirred. Samples were left stand for 30 min at 4°C, then centrifuged, and aliquots of supernatant were measured. Besides the control, samples were prepared by adding 0.13 M phosphate buffer (pH 7.4) instead of sera or HSA and measured as described.

Protein bindings were calculated as follows:
Protein binding = (1 − A / (K x T1)) x 100
T1 : radioactivity in the supernatant
T2 : radioactivity in a sample
A : charcoal adsorption radioactivity (T2-T1)
K : adsorption constant; calculation from the radioactivity measurement result in the control sample, K = A / T1

8. Determination of Radioactivity
1) Total Radioactivity
Ten–50 µl blood samples were dissolved in 1 ml of soluene–350 (Packard) and mixed with 10 ml of scintillators (ACS II, Amershams) after decolorization and neutralization. Then, 10–50 µl plasma samples, 500 µl urinary samples, 500 µl expired air samples and 200–300 µl toluene layer samples of carcass solution were mixed with 10 ml of ACS II. Two hundred–300 µl of fecal samples and 300 µl of the water layer samples of carcass so-
lution were combusted in a sample oxidizer (ASC-113; Aloka). The radioactivity in each sample was measured by liquid scintillation counter (LSC, LS3801 or LS6000; Beckman). Counting efficiency was assessed by the external standard method.

2) Volatile Radioactivity

Five hundred μl of urinary sample was lyophilized in the scintillation vial, residue was dissolved by 500 μl of distilled water, and then mixed with 10 ml of ACS II. Two hundred–300 μl fecal homogenate samples were lyophilized in a paper cup, 200 μl of distilled water was added to the lyophilized article and the samples were combusted in a sample oxidizer. The radioactivity (non-volatile radioactivity) in each sample was measured with the LSC. The volatile radioactivity was calculated by subtracting the nonvolatile radioactivity from the total radioactivity.

3) Immunoreactive Radioactivity in Plasma or Urine

One hundred μl of sample (plasma or urine) and 100 μl of 10 mM phosphate buffer saline (pH 7.4) containing 1 % BSA, 0.1% sodium azide (sample, buffer A) or 100 μl of standard (blank plasma or urine) and 100 μl of 3H–SK-896 standard solution (0.050, 0.10, 0.20, 0.50, 1.0, 2.0 and 5.0 ng/ml) were added to the assay tube. Then, 100 μl of rabbit anti-human motilin antiserum at 1/100 dilution with buffer A was added and incubated for 48 h at 4°C. Antibody bound 3H-SK-896 was precipitated using 100 μl of rabbit serum at 1/25 dilution and 100 μl of goat anti-rabbit IgG (Cappel) at 1/10 dilution. The tubes were incubated for 24 h at 4°C and centrifuged for 20 min at 3000 rpm. After the supernatant was aspirated, the precipitation was washed with 500 µl of buffer A and re-centrifuged. The precipitation was suspended by 200-300 µl fecal homogenate samples were added to the lyophilized article and the samples were combusted in a sample oxidizer. The radioactivity (non-volatile radioactivity) in each sample was measured with the LSC. The volatile radioactivity was calculated by subtracting the nonvolatile radioactivity from the total radioactivity.

9. Pharmacokinetic Data Analysis

The radioactivity level in plasma was expressed as ng equivalent of SK–896 per ml of plasma. The pharmacokinetic parameters were calculated from plasma immunoreactive concentration-time data. The elimination half-life (t1/2) of the elimination phase was estimated using the least square method of actual data. The area under the curve (AUC) and under the moment curve (AUMC) of concentration versus time were determined by the trapezoidal rule and by extrapolating to infinity. Plasma clearance (CLp), mean residence time (MRTmc) and volume of distribution at steady state (Vdss) were computed by noncompartmental methods.6) The Vdss following intravenous infusion was determined by the following equation: Vdss = dose × MRT/AUC–ko × T2/(2 × AUC), where ko and T are the infusion rate and infusion time, respectively.

10. Statistical Analysis

Statistical significance was performed using the one-way layout analysis of variance, Scheffe multiple comparison test and Aspin-Welch’s t-test. P values less than 0.05 were considered significant.

Results

1. Pharmacokinetic Studies

The time courses of blood and plasma levels of total radioactivity, plasma levels of immunoreactive radioactivity and pharmacokinetic parameters after intravenous bolus injection of male and female rats are shown in Fig. 1 and Table I, respectively. After intravenous bolus injection, blood and plasma levels of total radioactivity declined within 30–40 min and increased once again within 4–6 h after injection and thereafter decreased slowly. On the other hand, plasma levels of immunoreactive radioactivity declined bi-exponentially with the elimination half-lives (t1/2) of 11.4 min (male) and 13.0 min (female). No significant differences in pharmacokinetic parameters (AUC, MRT, CLp, Vdss and t1/2) were observed between male and female rats. There was no sex difference in pharmacokinetics after single intravenous bolus injection.

The time courses of blood and plasma levels of total radioactivity, plasma levels of immunoreactive radioactivity and pharmacokinetic parameters during and after intravenous infusion of male rats are shown in Fig. 2 and Table II, respectively. During constant rate intravenous infusion, blood and plasma levels of total radioactivity were increased rapidly. After stopping the infusion, blood and plasma levels of total radioactivity declined within 50 min–1 h, increased once again at 6–8 h, and thereafter decreased slowly. On the other hand, the plasma immunoreactive radioactivity level was increased rapidly during infusion, and declined bi-exponentially with the t1/2 of 7.80 min after stopping the infusion. No significant differences in pharmacokinetic parameters (CLp, Vdss and t1/2) were observed between intravenous bolus injection and constant rate intravenous infusion. There was no essential change in the pharmacokinetics by administration rate.

2. Excretion Studies

Cumulative excretions of total radioactivity in male and female rats after intravenous bolus injection are shown in Table III. Also, the ratios of the volatile radioactivity fraction to total radioactivity in urine and feces are shown in Table IV and V, respectively. Within 120 h after administration, 10.9%, 9.55% and 3.84% of administered radioactivity were excreted into urine, feces and expired air in male, and 22.7%, 13.1% and 5.38% in female rats, respectively. 73.2% of the dose
remained in the carcasses at 120 h post dosing in male, and 58.9% in female rats, respectively. The ratios of the volatile radioactivity to total radioactivity excreted in urine and feces increased by the time progress after administration. 96.0% and 61.5% of radioactivity excreted into urine and feces at 96-120 h after administration were the volatile fraction in male, and 98.8% and 68.5% in female rats, respectively. Also, the urinary excretion of immunoreactive radioactivity until 6 h was below the lower limit of determination (0.050 ng/ml). In female rats, the radioactivity excreted in urine, feces and expired air was 2.1, 1.4 and 1.4 times higher than by male rats.

The recovery of radioactivity in male and female rats remained in the carcasses at 120 h post dosing in male, and 58.9% in female rats, respectively. The ratios of the volatile radioactivity to total radioactivity excreted in urine and feces increased by the time progress after administration. 96.0% and 61.5% of radioactivity excreted into urine and feces at 96-120 h after administration were the volatile fraction in male, and 98.8% and 68.5% in female rats, respectively. Also, the urinary excretion of immunoreactive radioactivity until 6 h was below the lower limit of determination (0.050 ng/ml). In female rats, the radioactivity excreted in urine, feces and expired air was 2.1, 1.4 and 1.4 times higher than by male rats.

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### Table III
Cumulative excretions of radioactivity in urine, feces and expired air after single intravenous bolus injection of rats with $^3$H-SK-896 at the dose of 4 μg/kg

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Urine</th>
<th>Feces</th>
<th>Expired air</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.740 ± 0.107</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>6</td>
<td>1.47 ± 0.16</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>24</td>
<td>3.09 ± 0.05</td>
<td>2.63 ± 0.24</td>
<td>0.681 ± 0.057</td>
<td>6.58 ± 0.11</td>
</tr>
<tr>
<td>48</td>
<td>4.93 ± 0.06</td>
<td>5.05 ± 0.21</td>
<td>1.42 ± 0.03</td>
<td>11.8 ± 0.2</td>
</tr>
<tr>
<td>72</td>
<td>6.87 ± 0.13</td>
<td>7.00 ± 0.31</td>
<td>2.26 ± 0.04</td>
<td>16.5 ± 0.6</td>
</tr>
<tr>
<td>96</td>
<td>8.67 ± 0.19</td>
<td>8.62 ± 0.58</td>
<td>2.99 ± 0.08</td>
<td>20.6 ± 0.9</td>
</tr>
<tr>
<td>120</td>
<td>10.9 ± 0.1</td>
<td>9.55 ± 0.80</td>
<td>3.84 ± 0.02</td>
<td>24.5 ± 1.0</td>
</tr>
</tbody>
</table>

Residual radioactivity in carcass at 120 h: 73.2 ± 0.5% of dose (male), 58.9 ± 0.5% of dose (female).

Each value represents the mean ± S.E. of three rats.

### Table IV
Urinary excretions of radioactivity derived from total and volatile fraction after single intravenous bolus injection of rats with $^3$H-SK-896 at the dose of 4 μg/kg

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Total (T)</th>
<th>Volatile fraction (V)</th>
<th>V/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>0.740 ± 0.107</td>
<td>0.161 ± 0.080</td>
<td>19.2 ± 9.4</td>
</tr>
<tr>
<td>3-6</td>
<td>0.727 ± 0.086</td>
<td>0.381 ± 0.109</td>
<td>52.0 ± 14.1</td>
</tr>
<tr>
<td>6-24</td>
<td>1.63 ± 0.21</td>
<td>0.857 ± 0.315</td>
<td>49.5 ± 14.6</td>
</tr>
<tr>
<td>24-48</td>
<td>1.84 ± 0.01</td>
<td>1.65 ± 0.01</td>
<td>89.6 ± 0.2</td>
</tr>
<tr>
<td>48-72</td>
<td>1.95 ± 0.06</td>
<td>1.81 ± 0.06</td>
<td>92.9 ± 0.6</td>
</tr>
<tr>
<td>72-96</td>
<td>1.78 ± 0.16</td>
<td>1.65 ± 0.16</td>
<td>92.8 ± 1.2</td>
</tr>
<tr>
<td>96-120</td>
<td>2.29 ± 0.13</td>
<td>2.20 ± 0.13</td>
<td>96.0 ± 0.5</td>
</tr>
</tbody>
</table>

### Table V
Fecal excretions of radioactivity derived from total and volatile fraction after single intravenous bolus injection of rats with $^3$H-SK-896 at the dose of 4 μg/kg

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Total (T)</th>
<th>Volatile fraction (V)</th>
<th>V/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>0.740 ± 0.107</td>
<td>0.161 ± 0.080</td>
<td>19.2 ± 9.4</td>
</tr>
<tr>
<td>24-48</td>
<td>2.63 ± 0.24</td>
<td>0.690 ± 0.079</td>
<td>26.3 ± 2.4</td>
</tr>
<tr>
<td>48-72</td>
<td>2.42 ± 0.02</td>
<td>1.06 ± 0.05</td>
<td>43.8 ± 1.5</td>
</tr>
<tr>
<td>72-96</td>
<td>1.93 ± 0.40</td>
<td>1.07 ± 0.27</td>
<td>53.7 ± 3.5</td>
</tr>
<tr>
<td>96-120</td>
<td>1.64 ± 0.27</td>
<td>1.06 ± 0.22</td>
<td>63.4 ± 3.3</td>
</tr>
</tbody>
</table>

### 3. Protein Binding Studies
The protein binding rates of the rat, dogs and human serum and also HSA are shown in Table VI. Percentages of protein binding of $^3$H-SK-896 in male rats, female rats, male dogs, female dogs, male subjects and HSA were 93.6-96.2%, 94.0-96.6%, 92.1-94.2%, 91.4-93.6%, 96.2-97.0% and 84.2-87.0%, respectively. Concentration-dependency of $^3$H-SK-896 binding to serum proteins was not observed over the concentration range studied.

Each value represents the mean ± S.E. of three rats.

was 97.8 % and 100 % of the dose, respectively.
Table VI  Protein bindings of $^3$H-SK-896 to serum and human serum albumin (HSA)

<table>
<thead>
<tr>
<th>Concentration of $^3$H-SK-896 (ng eq./ml)</th>
<th>1.0</th>
<th>10</th>
<th>100</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein binding (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>97.0±0.1</td>
<td>96.2±0.0</td>
<td>96.4±0.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rat serum</td>
<td>96.2±0.0</td>
<td>93.7±0.1</td>
<td>93.6±0.1</td>
<td>94.5±0.2</td>
</tr>
<tr>
<td>female</td>
<td>96.6±0.1</td>
<td>94.8±0.1</td>
<td>94.0±0.1</td>
<td>94.8±0.1</td>
</tr>
<tr>
<td>Dog serum</td>
<td>94.2±0.0</td>
<td>92.1±0.1</td>
<td>92.5±0.3</td>
<td>92.7±0.5</td>
</tr>
<tr>
<td>female</td>
<td>93.6±0.1</td>
<td>91.4±0.1</td>
<td>92.4±0.1</td>
<td>92.5±0.2</td>
</tr>
<tr>
<td>HSA</td>
<td>86.3±0.7</td>
<td>84.2±0.3</td>
<td>85.3±0.1</td>
<td>87.0±0.1</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. (n=3). N.D.: not determined

Discussion

In the present study, the pharmacokinetics of SK-896 was investigated after single intravenous administrations of $^3$H-SK-896 to rats.

After single intravenous bolus injection of male and female rats or after single constant rate intravenous infusion to male rats, the disappearance of the immunoreactive radioactivity in plasma was fast and reached undetectable levels 2 h after administration. These results indicate that SK-896 was metabolized rapidly, and total radioactivity detected later than 2 h after administration was the radioactivity of metabolites without immunoreactivity or metabolites that were incorporated into the organism component.

When $^3$H-SK-896 was intravenously administered to rats, radioactivity was excreted very slowly since 73.2% and 58.9% of the dose remained in the male and female carcasses, respectively, at 120 h post dosing. This was conceivable not only due to late excretion of SK-896, but also because the metabolites incorporated into the organism components or metabolites as tritiated water remained in the body. This was supported by the fact that the rate of the volatile radioactivity fraction (the tritiated water) to total radioactivity of the urine and feces increased with time after administration.

On the other hand, in female rats, the radioactivity excreted in urine, feces and expired air was 2.1, 1.4 and 1.4 times higher, respectively, than in male rats. This sex difference, however, may not be owing to a difference in the excretion process of $^3$H-SK-896 so much as to a difference in the excretion process of tritiated water after a metabolite was recycled in the organism. In male and female rats, $^3$H-SK-896 or metabolite with immunoreactivity were not excreted into urine; most of the radioactivity excreted into urine 24 h after administration was a volatile fraction (tritiated water), and there was no sex difference in the pharmacokinetics of immunoreactive radioactivity in plasma.

The ratios of serum protein bindings of $^3$H-SK-896 in rat, dogs and human were above 92% at the range of 1.0–1000 ng/ml in vitro, indicating very strong binding of SK-896 to serum protein. The protein binding ratio of HSA is 84.2–87.0% and accounted for 88–89% of the binding rates of human serum, indicating SK-896 was mainly bound to serum albumin.

In conclusion, it was observed in this study that $^3$H-SK-896 would be metabolized and eliminated rapidly after intravenous administration. However, the excretion of total radioactivity was slow, presumably because the metabolite was recycled in the body. Also, binding to the serum protein of $^3$H-SK-896 was strong in rat, dog or human. Comparison with the protein binding ratio of HSA led us to infer that SK-896 was bound to serum albumin.

References